

REMARKS

The Specification has been amended to correct errors present in the nucleotide sequence of SEQ ID NOs: 3, 4, 5, 6, 7, and 8.

No new matter is added by these amendments. As is made clear from the Specification, each of these sequences describes a ribozyme. As would have been well known to a person of skill in the art at the time the application was filed, a ribozyme is an RNA molecule. See, *e.g.*, Oxford Dictionary of Biochemistry and Molecular Biology, Oxford University Press, 1997 at 576, a copy of which is attached hereto. RNA molecules (other than mRNA molecules) do not contain thymine ("T") residues, but rather uracil residues. See, *id.* at 647 and 672, copies of which are attached. Accordingly, a person of skill in the art would have understood that the RNA sequences represented by SEQ ID Nos: 3-8 would not contain thymine ("T") residues, but rather would contain uracil residues. Thus, the Amendment which is a mere correction of a typographical error, incorporates no new matter.

A copy of a corrected sequence listing (paper and computer readable form) is also enclosed. The corrected sequence listing contains amendments to SEQ ID Nos: 3-8, wherein the uracil residues have been substituted by thymine residues, to correct the typographical error. No new matter is incorporated by this amendment, for the reasons discussed above.

Also enclosed is a copy of the Notice to Comply with Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures, and a Statement under 37 CFR 1.821 *et seq.*, that the sequence listing presented in the paper form is identical to that submitted in the computer readable form.

CONCLUSION

In view of the foregoing, it is submitted that the application is fully compliant with all requirements for patent applications containing nucleotide sequence and/or amino acid sequence disclosures. Prompt examination and allowance of the claims is respectfully requested.

Respectfully submitted,

YASUHIKO TAKAHASHI, *et al.*

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(Date)

By:

KRISTYNE A. BULLOCK

Registration No. 42,371

AKIN GUMP STRAUSS HAUER & FELD LLP

One Commerce Square

2005 Market Street, Suite 2200

Philadelphia, PA 19103-7013

Telephone: 215-965-1200

Direct Dial: 215-965-1348

Facsimile: 215-965-1210

E-Mail: kbullock@akingump.com

KAB:cmb

7108423

Enclosures:

Oxford Dictionary of Biochemistry and Molecular Biology (pp. 576, 647, 672)

Statement under 37 CFR 1.821

Paper and Computer Readable Copies of Sequence Listing

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ribothymidyl

thymidylic acid is also an alternative recommended name for ribothymidine 5'-phosphate.

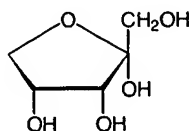
ribothymidyl the ribothymidine[mono]phospho group; the acyl group derived from **ribothymidylic acid**.

ribovirus an alternative name for **viroid**.

ribozyme an RNA molecule with catalytic activity. Such activity was first discovered by Sidney Altman in 1981 for **RNase P**. Thomas R. Cech in 1982 discovered the catalysis by RNA of the reactions involved in the **splicing** of ribosomal RNA from *Tetrahymena*. In this reaction a 414-nucleotide **intron** is removed from a 6.4 kb precursor to yield the mature 26S rRNA molecule. A guanosine nucleotide is essential in the reaction, binding to the RNA and then attacking the 5' splice site to form a phosphodiester bond with the 5' end of the intron. After further reactions the 414-nucleotide intron is released and undergoes two further splicing reactions to produce a linear RNA of 395 nucleotides which has lost 19 nucleotides and is named L19. L19 is catalytically active and is a true enzyme acting both as a nuclease and a polymerase. The rate of conversion of a pentacytidylate into longer and shorter oligomers is about 10^{10} times the uncatalysed rate. Mg^{2+} plays an essential role. Much smaller ribozymes have been demonstrated, e.g. the **viroids** which infect plants and undergo self-splicing after replication. Comparisons of nucleotide sequences in the vicinity of specific cleavage sites suggest that the active site has a **hammerhead** secondary structure consisting of three helical regions radiating from a central core of unpaired nucleotides. mRNA precursors in the mitochondria of yeast and fungi also undergo self-splicing as do some RNA precursors in chloroplasts. Such reactions can be classified according to the nature of the unit that attacks the upstream splice site. Group I are mediated by a guanosine cofactor. In group II the attacking moiety is the 2'-OH of a specific adenylate of the intron. Such reactions resemble those that occur in the **spliceosomes**. The work on ribozymes has a special interest in respect of theories on the origin of life because those interested in the origin of life on earth favour the idea that the first macromolecules were composed of RNA. The fact that RNA can catalyse reactions lends support to their views. See also **ribonuclease**.

(Rib5)ppA symbol for adenosinediphosphoribose (alternative to AdoPPRib or A5'pp5Rib).

D-ribulose a nonsystematic name for the ketopentose, D-erythro-2-pentulose.



α -D-furanose form

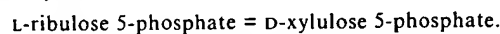
D-ribulose 1,5-bisphosphate *abbr.*: RBP or RuBP; *former name*: ribulose diphosphate (*abbr.*: RDP or RuDP); a key metabolite of the **reductive pentose phosphate cycle**. It is formed in a reaction in which ribulose 5-phosphate is phosphorylated by ATP, catalysed by phosphoribulokinase, EC 2.7.1.19, and is a substrate of **ribulose-bisphosphate carboxylase**.

ribulose-bisphosphate carboxylase EC 4.1.1.39; *systematic name*: 3-phospho-D-glycerate carboxy-lyase (dimerizing); *other names*: ribulose-bisphosphate carboxylase/oxygenase (*abbr.*: Rubisco); the key enzyme in the fixation of CO_2 in the **reductive pentose phosphate cycle**; a copper protein. One molecule of D-ribulose 1,5-bisphosphate reacts with one molecule of CO_2 to yield two molecules of 3-phospho-D-glycerate, which can be converted into a hexose product (fructose 6-phosphate). If, as is possible, D-ribulose 1,5-bisphosphate reacts with O_2 instead of CO_2 , the products are one molecule each of 3-phospho-D-glycerate and 2-phosphoglycolate. This

enzyme comprises up to 50% of leaf proteins and is the most abundant protein in the biosphere. Examples, all from tomato: large chain precursor: database code RBL_LYCES, 477 amino acids (52.62 kDa); small chain 1 precursor (less 17): database code RBS1_LYCES, 181 amino acids (20.28 kDa); small chain 2a precursor (less 5): database code RBS2_LYCES, 180 amino acids (20.25 kDa); small chain 3a/3c precursor: database code RBS3_LYCES, 180 amino acids (20.21 kDa); small chain 3b precursor: database code RBS4_LYCES, 180 amino acids (20.19 kDa).

D-ribulose 5-phosphate the 5-phosphate ester of D-ribulose; it is a component of the **pentose phosphate pathway**, the product of 6-phosphogluconate dehydrogenase. It is converted to ribulose 1,5-bisphosphate in the **reductive pentose phosphate cycle** of photosynthesis.

L-ribulose-phosphate 4-epimerase EC 5.1.3.4; an enzyme that catalyses the reaction:



Example: *araD* gene product from *Escherichia coli*: database code ARAD_ECOLI, 231 amino acids (25.49 kDa).

Richner-Ranhart syndrome see **tyrosine transaminase**.

ricin a highly toxic lectin obtained from the seeds of the castor oil plant, *Ricinus communis*. It is a 493 amino-acid glycoprotein dimer comprising an A chain (M_r 66 000) and a B chain (M_r 34 000) linked by a disulfide bond. Ricin binds to the cell via interaction between the B chain and galactose groups on cell-surface receptors; then the disulfide bond is cleaved allowing the A chain to enter the cell. The A chain then binds to the proteins of the 60S ribosomal subunit, apparently in the region of binding of elongation factors eEF-1 and eEF-2, thereby halting protein translation. Protein from *R. communis* (precursor): database code RIC1_RICCO, 576 amino acids (64.09 kDa). See also **ribosome-inactivating protein**.

rickets or **rachitis** a disturbance of calcium (and phosphate) metabolism in young growing animals (including humans), resulting from a deficiency of **vitamin D** or of its activation. It is characterized by softening of the bones and skeletal deformities, particularly bowing of the legs.—*rachitic adj.*

RID *abbr.* for radial immunodiffusion.

RIEP *abbr.* for rocket immunoelectrophoresis.

Rieske protein EC 1.10.99.1; *recommended name*: plastoquinol-plastocyanin reductase; *systematic name*: plastoquinol:oxidized-plastocyanin oxidoreductase; one of the four subunits of the cytochrome b_6-f complex. An enzyme that catalyses a reaction between plastoquinol-1 and two molecules of oxidized plastocyanin to form plastoquinone and two molecules of reduced plastocyanin. It is a high-potential 2Fe-2S protein. Example, precursor from tobacco: database code UCRI_TOBAC, 228 amino acids (24.12 kDa). Apart from the Rieske protein the subunits of the b_6f complex consist of **cytochromes b_6** and **f** and a 17 kDa peptide; it is similar to other iron-sulfur proteins (e.g. in mitochondria) and has three motifs.

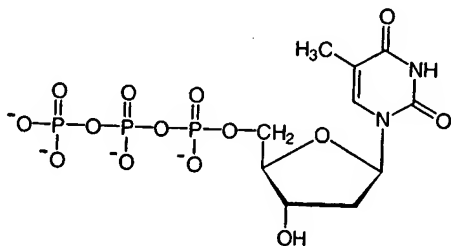
rifampicin or **rifampin** an antibiotic, M_r 823, obtained as a semisynthetic derivative of rifamycin B, from which it differs only in the substituents on the ring marked X in the structure for that compound. It acts by inhibiting bacterial transcription, and is effective in sensitive bacteria (see **rifamycin**) at extremely low concentrations ($0.01 \mu\text{g ml}^{-1}$) while having no effect on eukaryotic transcription at 10^4 times that dose. Mitochondrial and chloroplast transcription may be affected at higher doses. It is stable when stored dry at 4°C in the absence of light. In addition to its use as an antibiotic it has application in molecular biology as an agent for removing plasmids from bacteria in a process known as **plasmid curing**.

rifamycin any of a group of closely related anti-transcription antibiotics produced by *Streptomyces mediterranei* that are active against Gram-positive bacteria and *Mycobacterium tuberculosis* but are much less effective against Gram-negative organisms. Chemically and functionally they are closely re-

is synthesized (very indirectly) from **uridine 5'-phosphate**, its immediate precursor being 2'-deoxyuridine 5'-phosphate – see **thymidylate synthase**. In addition, resynthesis of dTMP from free thymidine formed by degradation of DNA can be effected via a **salvage pathway**.

thymidine phosphorylase see **pyrimidine-nucleoside phosphorylase**.

thymidine 5'-triphosphate symbol: dThdS'PPP or pppdT; the recommended name for thymidine triphosphate (abbr.: dTTP), 5'-triphosphothymidine, 5'-thymidyl diphosphate, thymidine 5'-(tetrahydrogen triphosphate); a substrate for DNA synthesis. It is formed from thymidine 5'-phosphate by the successive action of nucleoside-phosphate kinase (EC 2.7.4.4) and nucleoside-diphosphate kinase (EC 2.7.4.6), which transfer in turn the terminal phosphoric residues from two molecules of adenosine 5'-triphosphate.



thymidyl either of the chemical groups formed by the loss of a 3'- or a 5'-hydroxyl group from the deoxyribose moiety of thymidine.

thymidylate 1 either the monoanion or the dianion of thymidylic acid. 2 any mixture of thymidylic acid and its anions. 3 any salt or ester of thymidylic acid.

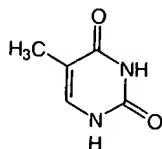
thymidylate synthase EC 2.1.1.45; systematic name: 5,10-methylenetetrahydrofolate:dUMP C-methyltransferase; an enzyme responsible for the formation of thymidylate, dTMP, by the *de novo* pathway. It catalyses reductive methylation of 2'-deoxy-5'-uridylate, dUMP, by reaction with 5,10-methylenetetrahydrofolate to form dihydrofolate and dTMP. (The dihydrofolate thus formed is reduced again to tetrahydrofolate by **dihydrofolate reductase**.) Thymidylate synthase is important as a target for the antineoplastic agents **fluorouracil** and **fluorodeoxyuridine**. These drugs are metabolized to fluorodeoxyuridylate, which is a potent irreversible inhibitor of the enzyme. Example from *Escherichia coli* (homodimer); database code TYSY_ECOLI, 264 amino acids (30.44 kDa); 3-D structure known; five motifs. See also **thymineless death**.

thymidylic acid the trivial name for any phosphoric monoester of thymidine. The position of the phosphoric residue on the deoxyribose moiety of a given ester may be specified by a prefixed locant – see **thymidine phosphate**. However, 5'-thymidylic acid is the ester commonly denoted, its locant usually being omitted if no ambiguity may arise. 5'-Thymidylic acid is also an alternative recommended name for **thymidine 5'-phosphate**.

thymidyl the thymidine[mono]phospho group; the acyl group derived from **thymidylic acid**.

thymine a former name for **thymopietin**.

thymine symbol: T or Thy; 5-methyluracil; 2,4-dihydroxy-5-methylpyrimidine; one of the two major pyrimidine bases present (as thymidine) in DNA but not found in RNA other than (as ribothymidine) in transfer RNA, where it is a minor base.



thymine dimer a pyrimidine dimer formed within a DNA strand from two adjacent thymine residues by **photodimerization**. It is most commonly of the cyclobutadipyrimidine type.

thymineless death the death of an animal or bacterial cell following a period of thymine (or thymidylate) deprivation. Although such a deprived cell may continue to produce protein and RNA, it can no longer synthesize DNA in the absence of thymidylate, and it eventually loses its vitality and dies, perhaps because it is also unable to repair altered or damaged DNA. The phenomenon may be demonstrated with a thymine-requiring auxotrophic bacterial mutant by omitting thymine from the suspending medium, or with any cell by adding **fluorouracil** or its metabolite **fluorodeoxyuridine**, with consequent inhibition of **thymidylate synthase**. A rapidly metabolizing animal cell, such as a tumour cell, is particularly susceptible to thymineless death.

thymine deoxyriboside an alternative name for **thymidine**.

thymine riboside an alternative name for **ribothymidine**.

thymineose a former name for 2-deoxy-D-ribose (see **deoxyribose**).

thymo+ comb. form of, or pertaining to, the thymus.

thymocyte (sometimes) any **lymphocyte** found in the thymus.

thymocyte mitogenic factor abbr.: TMF; an alternative name for **interleukin 2**.

thymocyte-stimulating factor abbr.: TSF; an alternative name for **interleukin 2**.

thymoma a tumour of thymic tissue.

thymonuclease another name for **deoxyribonuclease I**.

thymonucleic acid an alternative term for **thymus nucleic acid**.

thymopentin a synthetic pentapeptide that corresponds to residues 32–36 of **thymopietin**.

thymopietin abbr.: TP; any one of three polypeptide hormones that result from alternative splicing of the same gene, and originally isolated from bovine thymus. They have pleiotropic actions on prothymocytes, inducing expression of the differentiation antigens such as **Thy-1**, and are important in T-cell development and function. They were discovered in studies on myasthenia gravis and impair neuromuscular transmission after injection in mice, due to an effect on the nicotinic acetylcholine receptor. A synthetic pentapeptide corresponding to positions 32–36, named **thymopentin** and with the sequence Arg-Lys-Asp³⁴-Val-Tyr, has full activity. **Splenin**, a corresponding peptide in spleen, contains a similar pentapeptide, called **splenopentin**; bovine splenin has Glu in place of Asp³⁴, and human splenin has Ala in the corresponding position. These replacements affect the biological activity. **Thymopietin** was formerly known as **thymine**.

thymosin any of several related polypeptide hormones of thymic origin that are involved in differentiation of T lymphocytes in the thymus. They include α_1 and β_4 thymosins, which are of known structure. **Thymosin β_4** (human, bovine, rat) has the sequence SDKPDMAIEKFDKSKLKKTTETQEKNP-PSKETIEQEKQAGES.

thymulin a zinc-requiring immunomodulatory thymic nonapeptide hormone known to induce intra- and extra-thymic T-cell differentiation. Its level is decreased in immunodeficiency and in autoimmune disease.

thymus (gland) a bilobed glandular organ located in the posteroventral part of the neck. In mammals the thymus is encapsulated and divided into lobules, each lobule consisting of cortex and medulla; the cortex consists mainly of lymphocytes (thymocytes). The thymus is responsible for populating the blood, lymph, and thymus-dependent areas with **T lymphocytes** during the neonatal period. The thymus is large at birth but diminishes in size with increasing age and may be difficult to identify in adults. It is important in **cell-mediated immunity**. — **thymic** adj.

thymus nucleic acid a former name for **deoxyribonucleic acid**. It was so termed because the thymus gland was found to be a particularly rich source of nucleic acid of a type that was thought at the time to be characteristic of animal tissues and

univalent 1 or **mónovalent** (of a chemical species) having a **valency** of one. **2** an unpaired chromosome seen during meiosis when bivalents are also present. Such a chromosome either lacks a homologue or results from **asynapsis**. —**univalency** *n*.

universal buffer mixture any solution containing several buffering systems chosen so as to provide a relatively high buffer capacity over a wide pH range.

universal donor an individual with O-type blood; such blood may be transfused into any recipient. *See* **ABO system**.

universal recipient an individual with AB-type blood; such individuals may be transfused with blood from any donor. *See* **ABO system**.

UNIX an operating system implemented on many current workstations and large computers.

unmediated transport or **nonmediated transport** the movement of a solute across a barrier by **diffusion** (def. 1).

unpaired electron an electron in an atom or molecule whose spin is not paired with the oppositely directed spin of another electron in the same atom or molecule. Only systems containing unpaired electrons will give an electron spin resonance signal. *See* **electron spin resonance spectroscopy**.

unsaponifiable lipid or **unsaponifiable material** part of a lipid sample that is not solubilized by **saponification**. It consists principally of steroids and terpenes.

unsaturated 1 (of a solution) able to dissolve more of the solute in question. **2** (of an organic compound) containing double bonds; *see also* **polyunsaturated**. **3** (of any chemical system, e.g. an enzyme or antibody) not fully saturated with ligand (e.g. substrate or antigen).

unsaturated-fatty-acid oxidation the degradation of unsaturated fatty acids to CO₂ and H₂O; this is achieved in large part by enzymes of the **beta-oxidation system**, but at certain points enzymes specific for unsaturated fatty acids are required. The first of these occurs when beta-oxidation reactions have removed carbons to the point at which the resulting acyl-CoA molecule has a Δ^3 -*cis* double bond; this is not a substrate for **enoyl-CoA hydratase**, but an **enoyl-CoA isomerase** (*see* **dodecenoyl-CoA Δ -isomerase**) converts it to a substrate with a Δ^2 -*trans* double bond. Another problem occurs when an acyl-CoA with a Δ^4 -*cis* double bond is produced, which again is not a substrate for **enoyl-CoA hydratase**, but the problem is solved by the action of **2,4-dienoyl-CoA reductase**, followed by **enoyl-CoA isomerase**; this combination yields the substrate with a Δ^2 -*trans* double bond.

untranscribed spacer a region of genomic DNA, lying between genes or groups of genes, that is not transcribed. *See* **spacer** (def. 1).

untranslated region any part of an mRNA molecule not coding for a protein; e.g. in eukaryotes the **poly(A) tail**.

unwinding protein any of the proteins concerned in DNA replication that have a higher affinity for single-stranded DNA than for double-stranded DNA and act to hold the strands apart during replication.

UPA *abbr.* for urokinase-type plasminogen activator; *see* **plasminogen activator**.

U5'pp1Gal *symbol for* uridinediphosphogalactose (alternative to **UrdPPGal** or **Urd-5'PP-Gal**).

U5'pp1Glc *symbol for* uridinediphosphoglucose (alternative to **UrdPPGlc** or **Urd-5'PP-Glc**).

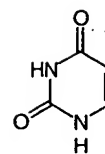
up-promoter a mutation that increases **promoter** strength in the expression of a gene.

upsilon *symbol:* υ (lower case) or Υ (upper case); the twentieth letter of the Greek alphabet.

upstream in or towards positions in a DNA molecule lying in the 5' direction relative to the start of transcription of a gene.

Ura *symbol for* a residue of the pyrimidine base **uracil**.

uracil *symbol:* Ura or (sometimes) U (not recommended); 2,4-dioxypyrimidine; 2,4-pyrimidinediol; one of the pyrimidine bases occurring in RNA (but not in DNA).



uracil

uracil-DNA N-glycosylase an enzyme (sub-subclass EC 3.2.2) that removes from DNA any uracil resulting from misincorporation of dUTP that has escaped the action of dUTPase (EC 3.6.1.23, dUTP pyrophosphatase). The enzyme hydrolyses the bond at C-1 of the deoxyribose to yield free uracil and DNA containing an apyrimidinic site; another enzyme, **apyrimidine endonuclease**, recognizes this site and cleaves the phosphodiester bond on the 5' side of the deoxyribose moiety. **Uracil-DNA N-glycosylase** may represent the evolutionary basis for the presence of T (not U) in DNA. Cytosine deamination leads to U residues, which would generate transition mutations on replication. In *Escherichia coli* the enzyme is the product of the *ung* gene and mutations in this can be used in certain forced site-directed mutagenesis protocols. The enzyme is a monomer, and is highly conserved in all species. Example, the product of the *ung* gene: database code UNG_ECOLI, 228 amino acids (25.53 kDa).

urate oxidase EC 1.7.3.3; *systematic name:* urate:oxygen oxidoreductase; *other name:* uricase. An enzyme that catalyses the reaction: urate + O₂ → intermediate(s) that lead(s) to **allantoin**. It is a conserved (five motifs) peroxisomal enzyme and a copper protein. Some mammals (e.g. humans, gorillas, etc.) that lack the enzyme are prone to **gout**. Example from rat: database code URIC_RAT, 302 amino acids (34.76 kDa).

Urd *symbol for* a residue of the ribonucleoside **uridine** (alternative to U).

UrdP *symbol for* any uridine phosphate.

Urd2'P *symbol for* uridine 2'-phosphate.

Urd-2'3'-P *symbol for* uridine 2',3'-phosphate.

Urd3'P *symbol for* uridine 3'-phosphate.

Urd-3'5'-P *symbol for* uridine 3',5'-phosphate.

Urd5'P *symbol for* uridine 5'-phosphate.

Urd5'PP *symbol for* uridine 5'-diphosphate (alternative to ppU).

UrdPPGal or **Urd-5'PP-Gal** *symbol for* uridinediphosphogalactose (alternatives to U5'pp1Gal).

UrdPPGlc or **Urd-5'PP-Glc** *symbol for* uridinediphosphoglucose (alternatives to U5'pp1Glc).

Urd5'PPP *symbol for* uridine 5'-triphosphate (alternative to pppU).

urea the water-soluble compound H₂N-CO-NH₂, produced in the liver via the **ornithine-urea cycle**. It is the main nitrogen-containing (urinary) excretion product in **ureotelic** animals. It is used as source of nonprotein nitrogen in ruminant livestock feeds.

urea cycle *see* **ornithine-urea cycle**.

urease EC 3.5.1.5; *systematic name:* urea amidohydrolase; a nickel-protein enzyme that catalyses the hydrolysis of urea to carbon dioxide and ammonia. A carbamoylated lysine provides an oxygen ligand to each nickel, thus explaining a requirement for CO₂ as an activator of urease apoenzyme. Example from *Proteus vulgaris*; a decamer of three subunits (probably in two $\alpha\beta_2\gamma_2$ complexes); α subunit: database code URE1_PROVU, 567 amino acids (60.86 kDa); β subunit: database code URE2_PROVU, 108 amino acids (12.14 kDa); γ subunit: database code URE3_PROVU, 100 amino acids (10.94 kDa). The 3-D structure is known for the *Klebsiella aerogenes* enzyme.